AF/1652

Docket No. 10347 (GS435/111656) PATENT APPEAL

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Date: June 30, 2003

Sheila Chang
Sheila Chang

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)

Heinrich BACHMANN *et al.*) Examiner: Yong Pak

Serial No.: 09/504,393) Group Art Unit: 1652

Filed: February 15, 2000)

For: **β,β-CAROTENE 15, 15'-DIOXYGENASES, NUCLEIC ACID SEQUENCES CODING THEREFOR AND THEIR USE**)

New York, New York
June 30, 2003

REQUEST FOR EXTENSION OF TIME, REQUEST FOR REINSTATEMENT OF APPEAL AND APPELLANTS' SUPPLEMENTAL BRIEF ON APPEAL

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Arlington, VA 22313-1450

Sir:

Pursuant to Rule 193(b)(2)(ii) and Paper No. 19 (at p. 2), reinstatement of the appeal is hereby requested. In view of this request for reinstatement of the appeal, submitted herewith is a Supplemental Brief On Appeal from the rejection of all claims pending and under examination in the above-identified application. Because the claims

have been twice rejected, this supplemental appeal is proper, even though the present rejection is non-final. *See* 37 CFR §1.191(a).

In accordance with 37 CFR §1.192(a), this brief is being submitted in triplicate.

The shortened statutory period of time for response to the Office Action of December 31, 2002, expired on March 31, 2003. A three-month extension of time to respond to the Office Action is hereby requested. With this extension, the due date for reply to the Office Action is June 30, 2003, with an executed certificate of mailing. 37 CFR §§1.8 and 1.136. Enclosed is a check in the amount of $930.00 to cover the fee for the extension of time. No other fee in connection with this Supplemental Brief On Appeal is believed due. If, however, any additional fee(s) are due, please charge any required fee not otherwise paid by check to Deposit Account No. 02-4467. A duplicate copy of this sheet is enclosed.

IDENTIFICATION OF REAL PARTY IN INTEREST

The real party in interest is ROCHE VITAMINS INC., which is the assignee of record of the present application and is a corporation organized and existing under and by virtue of the laws of the State of Delaware. Ownership of ROCHE VITAMINS INC. lies in F. HOFFMANN-LA ROCHE AG., a company organized and existing under the laws of the Swiss Confederation.

RELATED APPEALS AND INTERFERENCES

Upon information and belief of the undersigned counsel, Appellants and the assignee of record are not aware that there are any pending appeals or

have been twice rejected, this supplemental appeal is proper, even though the present rejection is non-final. *See* 37 CFR §1.191(a).

In accordance with 37 CFR §1.192(a), this brief is being submitted in triplicate.

The shortened statutory period of time for response to the Office Action of December 31, 2002, expired on March 31, 2003. A three-month extension of time to respond to the Office Action is hereby requested. With this extension, the due date for reply to the Office Action is June 30, 2003, with an executed certificate of mailing. 37 CFR §§1.8 and 1.136. Enclosed is a check in the amount of $930.00 to cover the fee for the extension of time. No other fee in connection with this Supplemental Brief On Appeal is believed due. If, however, any additional fee(s) are due, please charge any required fee not otherwise paid by check to Deposit Account No. 02-4467. A duplicate copy of this sheet is enclosed.

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RELATED APPEALS AND INTERFERENCES

Upon information and belief of the undersigned counsel, Appellants and the assignee of record are not aware that there are any pending appeals or

interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

STATUS OF ALL CLAIMS AND AMENDMENTS

A. Status Prior To Final Rejection

As filed, this Application contained claims 1-35.[1/] The claims were subject to a four-way restriction requirement. (Paper No. 8, pp. 2-4). On December 29, 2000, an election was made to prosecute the subject matter of Group II (claims 6-15, 19-32, and 34-36). In Paper No. 8, the Examiner withdrew claims 1-5, 16-18, and 33 from consideration and allowed claims 28-32 and 34-36. (*Id.* at p.1, ¶¶ 4a and 5). In the RESPONSE TO OFFICE ACTION INCLUDING AMENDMENT AND PETITION FOR EXTENSION OF TIME dated July 31, 2001 ("July 31, 2001 Response"), the election was affirmed. (*See* p. 7, lns. 18-19).

In preparing the reply to Paper No. 8, Appellants became aware that the polypeptide identified in the specification as SEQ ID NO:1 had been misnamed. This information was promptly brought to the attention of the Examiner and claims 6, 8, 9, 11-15, 19, 27, 28, and 34-36 were subsequently amended (1) in view of the restriction requirement and (2) so that they no longer recited or depended from a claim that recited "β,β-carotene 15, 15'-dioxygenase." (July 31, 2001 Response, pp. 2-4).

No further amendments were presented prior to final rejection.

1/ As originally filed, the application contained two claims numbered "27." By Examiner's Amendment, beginning with the second occurrence of claim 27, the claims were renumbered 28-36. (Paper No. 8, p. 2, lns. 2-5).

B. Status After Final Rejection

Amendments were presented to the claims and claims were added after final rejection, but the amendments and added claims were not entered. (*See* RESPONSE TO OFFICE ACTION INCLUDING AMENDMENT AND PETITION FOR EXTENSION OF TIME dated February 28, 2002 ("February 28, 2002 Response") and Paper No. 14, p. 2, lns. 2-5). In a SUPPLEMENTAL AMENDMENT AFTER FINAL REJECTION filed on October 2, 2002, claims 7-9 were cancelled, without prejudice, to reduce the issues on appeal. The Supplemental Amendment was entered by the Examiner. (Paper No. 17).

A Brief On Appeal was filed on October 11, 2002 ("Appeal Brief 1") appealing the rejections of claims 6, 10-15, 19-32, and 34-36 under 35 USC §101 (utility) and §112, first paragraph (enablement).

A continuation-in-part application was filed on January 15, 2002. This application was assigned U.S. Serial No. 10/053,192. A Response To Restriction Requirement was filed on June 20, 2003. No further substantive examination has occurred in the '192 application.

C. Status After Appeal

The Examiner reopened prosecution and issued a non-final Office Action (Paper No. 19) with "new grounds of rejection" in response to Appeal Brief 1.

No further amendments have been presented subsequent to Paper No. 19.

D. **Identification Of Claims On Appeal**

Claims 6, 10-15, 19-32, and 34-36 are on appeal and are reproduced in APPENDIX I to this brief.

SUMMARY OF THE INVENTION AND THE CLAIMS

Vitamin A is a biologically active substance essential for man and animals. The term "vitamin A" embraces a class of compounds that includes retinal, retinol, 3-dehydroretinol, retinoic acid, isomers of such compounds, and retinylesters. (Specification, p. 1, lns. 14-16).

Vitamin A is formed by converting precursor carotenoids (also called provitamins A) into vitamin A. These carotenoid precursors (including β-carotene) can only be formed in plants, in photosynthetically active microorganisms, and in other microorganisms. Man and animals are able to convert such provitamins A enzymatically into vitamin A. (Specification, p. 5, lns. 13-17).

Thus, attempts have been made to commercially produce vitamin A from carotenoid precursors for many years. The first step in the process was to purify and characterize the enzymes responsible for converting, *e.g.* β-carotene into retinal. Since 1955, many attempts have been made to purify and characterize these enzymes using biochemical methods. (Specification, p. 6, lns. 3-8).

The prior attempts to form retinal (a form of vitamin A) from carotenoid precursors have a number of disadvantages. Most notably, the prior attempts have purified enzymes having specific activities of no more than 600 pmol retinal formed/mg protein per hour. These specific activities are not commercially useful, and thus have been considered failed attempts. (Specification, p.6, lns. 5-10).

Accordingly, the present invention provides a process for enzymatically converting β-carotene to retinal through the use of an enzyme identified and purified using a combination of biochemical and molecular biological techniques. Using these techniques, a chicken enzyme was purified 226-fold, yielding a specific activity over four times higher than previously reported, *i.e.*, 2,500 pmol/h/mg. From this preparation, a partial amino acid sequence was obtained, which was used to make degenerate PCR primers. (Specification, p. 6, lns. 12-21).

Using the PCR primers, a full length cDNA was identified, cloned, and sequenced. (Specification p. 7, lns. 5-13 and p. 22, lns. 17-19). A recombinantly expressed protein encoded by the cDNA was shown to enzymatically cleave β-carotene to retinal. (Specification p. 25, lns. 9-12 and Figs. 7 and 8).

STATEMENT OF THE REJECTIONS AND ISSUES

Whether claims 6, 10-15, 19-32, and 34-36 are unpatentable under 35 USC §112, first paragraph for failing to describe the claimed subject matter in the specification in such a way to convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention.

Whether claims 6, 10-15, 19-32, and 34-36 are unpatentable under 35 USC §112, first paragraph for failing to teach how to make and/or use the claimed invention.

GROUPING OF CLAIMS

Not all claims stand or fall together.

Arguments are presented below, which demonstrate the patentability of claims 6, 10, 11, and 28-32.

Separate arguments are presented, which demonstrate the separate patentability of claims 12-15 and 34-36.

Separate arguments are presented, which demonstrate the separate patentability of claims 19-27.

SUMMARY OF THE POSITIONS TAKEN BY THE EXAMINER IN THE FINAL OFFICE ACTION

After the first Office Action was issued (Paper No. 8), Appellants determined that the name given to the polypeptide designated "SEQ ID NO:1" in the specification, namely "β,β-15,15' dioxygenase" was incorrect. This misnaming was identified by the Appellants through subsequent studies of the polypeptide using a more sensitive assay that was developed after the filing date of the application whereby symmetric vs. non-symmetric metabolites of β,β-carotene could be resolved. These subsequent studies revealed that the enzyme disclosed in the application is a β,β-carotene 15, 15'-monooxygenase. (*See* Michele G. Leuenberger *et al.*, "The Reaction Mechanism of the Enzyme-Catalyzed Central Cleavage of β-Carotene to Retinal," *Angew. Chem. Int. Ed.* 2001, 40, No. 14, pp. 2614-2617, attached hereto as Appendix II). All other physical and structural properties disclosed in the application, including the substrate, reaction product, and structures of the nucleotide and polypeptide sequences, are correct. (*See Id.*; and July 31, 2001 Response, p. 4, lns. 18-19).

This information was promptly conveyed to the Examiner and the Examiner's supervisor (Dr. Ponnathapura Achutamurthy) during a series of teleconferences prior to, and again in, the July 31, 2001 Response.

In the Final Action, the Examiner rejected claims 6-36 under 35 USC §101 allegedly because the claimed invention "lacks patentable utility."[2/] (Paper No. 11, p. 3, lns. 1-2). In making the rejection, the Examiner asserted that "the claimed polynucleotides are not supported by either a specific and substantial asserted utility." (*Id.*, lns. 2-3). The Examiner further asserted that "the specification does not teach the function of SEQ ID NO:1." (*Id.*, lns. 8-9).

The Examiner also asserted that "the β,β-carotene 15,15'-monooxygenase activity of SEQ ID NO:1 is unpredictable in the art because SEQ ID NO:1 has homology with polypeptides with β,β-carotene 15,15'-dioxygenase activity" and that "[t]hese claims amount to a polypeptide with unknown function and a polypeptide with unknown and unpredictable function has no utility." (*Id.*, lns. 11-13).

The Examiner then concluded that "[t]herefore, there is no specific, substantial, or credible utility that is well known, apparent, or implied by the relationship of the instant polynucleotide to the polynucleotide encoding SEQ ID NO:1." (*Id.*, lns. 13-15).

The Examiner further rejected "claims 6-36"[3/] under the enablement provision of 35 USC §112, first paragraph. (Paper No. 11, pp. 3-4). In making the rejection, the Examiner asserted that "[s]ince the claimed invention is not supported by

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2/ In making the rejection, the Examiner ignored the fact that she had previously ***withdrawn*** claims 16-18 and 33 (in addition to claims 1-5) from consideration and allowed claims 28-32 and 34-36. This error was subsequently corrected in the current Office Action, Paper No. 19.

3/ *See* note 2, *supra*.

either a specific asserted utility or a well established utility ... one skilled in the art clearly would not know ***how to use*** the claimed invention so that it would operate as intended without ***undue experimentation***." (*Id.* at p. 4) (emphasis added).

The Examiner also asserted that "the specification does not teach the function of the polypeptide encoded by SEQ ID NO:2 because the specification does not teach the correct function of the polypeptide. In the state of the art, the function of a polypeptide is unpredictable from its structure and the functionality of a polypeptide must be known in order to use the polypeptide." The Examiner concluded, "[t]herefore, the specification does not teach ***how to use*** SEQ ID NO:1 and DNA molecules encoding SEQ ID NO:1 without ***undue experimentation***." (*Id.*) (emphasis added).

In the Advisory Action, in response to Appellant's showing that:

> (i) The specification describes at least three utilities for the claimed invention;
>
> (ii) That a compound and all of its properties are inseparable and that a name alone does not impart or depart utility; and
>
> (iii) That only the name of the enzyme was misidentified in the specification, ***not*** its function, *i.e.*, cleavage of β-carotene to retinal;

the Examiner asserted that "[e]xamples 1-6 all relate to detecting activity, cloning, purification and expression of β,β-carotene 15,15'-dioxygenase" and that "[t]he specification does not support utility for a β,β-carotene 15,15'-monooxygenase." The Examiner then summarily contended that "[i]dentifying a polypeptide as a β,β-carotene 15,15'-monooxygenase does not endow the polypeptide with such a utility. ... [nor does the specification disclose] ***how to use*** SEQ ID NO:1 and 3 or DNA molecules encoding

SEQ ID NO:2 without ***undu experimentation***." (*See* Paper No. 14, p. 3, lns. 7-11 and p. 4, lns. 2-6) (emphasis added).

SUMMARY OF THE POSITIONS TAKEN BY THE EXAMINER UPON REOPENING PROSECUTION

In response to Appellants' uncontested demonstration in Appeal Brief 1 that:

(i) The polynucleotide and polypeptide sequences, the identified substrate for SEQ ID NO:1 (β-carotene) and the reaction product (retinal) were correctly disclosed in the specification;

(ii) That the specification discloses at least three functions/uses for the claimed polynucleotide (SEQ ID NO:2) and the polypeptide it encodes (SEQ ID NO:1) including as a participant in the pathway leading to production of vitamin A, cleavage of carotene molecules, and production of transgenic plants; and

(iii) That only the name of the enzyme was misidentified in the specification, ***not*** its function, *i.e.*, cleavage of β-carotene to retinal,

the Examiner declined to go forward with the appeal; instead withdrawing both pending rejections, reopening prosecution and issuing a non-final Office Action. (Paper No. 19, p. 2, lns. 9-10 and p. 3, lns. 1-2). The Examiner characterized the Office Action as containing "new grounds of rejection." (*Id.*, p. 2, ln. 10).

In the first so-called "new ground of rejection," the Examiner rejected claims 6, 10-15, 19-32, and 34-36 under 35 USC §112, first paragraph "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." (*Id.*, p. 6, lns. 1-5).

In making the rejection, the Examiner acknowledged that the claims "are drawn to DNA encoding a polypeptide of SEQ ID NO:1." (*Id.*, ln. 6). The Examiner, however, asserted that "[e]ven though both enzymes [*i.e.*, mono- and di-oxygenases] transform carotene to retinal, each reaction is different ...." (*Id.*, lns. 16-17). The Examiner further asserted that "[t]he specification only describes ***how to use*** the claimed invention as a dioxygenase and not as a monooxygenase. Also the specification only describes assaying a polypeptide for β,β-carotene 15,15'-dioxygenase activity and not for β,β-carotene 15,15'-monooxygenase activity." (*Id.*, lns. 19-22) (emphasis added). The Examiner then concluded one "would not recognize from the specification that applicants were in possession of a polypeptide with β,β-carotene 15,15'-monooxygenase activity." (Paper No. 19 at 6-7).

In the second so-called "new ground of rejection," the Examiner rejected claims 6, 10-15, 19-32 and 34-36 under 35 USC §112, first paragraph "as containing subject matter which was not described in the specification in such a way as to enable one ... to make and/or use the invention." (*Id.*, p. 3, lns. 9-12). In making the rejection, the Examiner asserted that "[t]he claims are drawn to DNA encoding a polypeptide of SEQ ID NO:1" but that "[t]he specification only teaches how to determine a polypeptide as being a β,β-carotene 15,15'-dioxygenase (Example 1) and the specification only teaches how to use a β,β-carotene 15,15'-dioxygenase." (*Id.*, lns. 20-23).

The Examiner also asserted that the "specification does not teach that SEQ ID NO:1 is a β,β-carotene 15,15'-monooxygenase nor how to use SEQ ID NO:1 as a β,β-carotene 15,15'-monooxygenase." (*Id.*, p. 4, lns. 5-7). The Examiner further asserted that "[e]ven though both enzymes [mono- and di-oxygenases] transform

carotene to retinal, each reaction is different" and that "the two enzymes are not exchangeable without altering the reaction mixture and the specification only teaches the use of a dioxygenase." (*Id.*, p. 4, ln. 18 – p. 5, ln. 6).

The Examiner concluded that the "breadth of the claims is much larger than the scope enable[d] by the specification;" that even though monooxygenases are "well known in the art;" one would "require guidance in order to use SEQ ID NO:1 in a manner reasonabl[y] correlated with the scope of the claims;" and that "without such guidance, the experimentation left to those skilled in the art is undue." (*Id.*, p. 4, lns. 6-7 and p. 5, lns. 14-21).

THE LEGAL STANDARDS

A. 35 USC §112, First Paragraph – Written Description

The patent statute requires that the "specification shall contain a written description of the invention," set forth in "full, clear, concise and exact terms." 35 USC §112, first paragraph. This written description requirement is separate and distinct from the statutory enablement requirement. *In re Barker*, 559 F.2d 588, 593, 194 USPQ 470, 474 (CCPA 1977).

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can clearly conclude that the inventor invented the claimed subject matter, *i.e.*, that the inventor had possession of the claimed invention. *MOBA, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1320, 66 USPQ2d 1429, 1439 (Fed. Cir. 2003); *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997); *In re Gostelli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989); *Fiers v Revel*,

984 F.2d 1164, 1170, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993); and *Regents of the University of California v. Eli Lilly and Co.*, 119 F.3d 1559, 1566, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997).

Compliance with the written description requirement of the statute is a question of fact. *In re Wertheim*, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). During prosecution, the Examiner has the burden of establishing a *prima facie* case, by a preponderance of the evidence, as to why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention recited by the claims. *In re Wertheim*, 541 F.2d at 263, 191 USPQ at 97 and *Ex parte Chen*, 2002 WL 87963, *3 (unpublished) (BPAI 2002).

The PTO has promulgated guidelines to be followed by Examiners in the evaluation of patent applications for compliance with the written description requirement of 35 USC §112, first paragraph. These guidelines do not have the force of law, but are based on the PTO's understanding of binding legal authority in this area. *See* MPEP §2163, 8th Ed. (Feb. 2003 Rev. 1) at 2100-158.

Thus, legal precedent and the PTO's own internal rules require that an Examiner:

(1) read and analyze the specification for compliance with the written description requirement;

(2) determine what each claim means; and

(3) compare the scope of each claim to the description in the application to determine if the applicant has demonstrated possession of the claimed subject matter.

(*See Id.* at 2100-159-165; *In re Wertheim*, 541 F.2d at 262, 191 USPQ at 96-97; *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972); and *In re Morris*, 127 F.3d 1048, 1053-1054, 44 USPQ 1023, 1027 (Fed. Cir. 1997)).

An applicant may show possession of a claimed invention in many ways, including through disclosure of drawings or structural chemical formulas that are sufficiently detailed to show that the applicant was in possession of the claimed invention as a whole. *Vas-Cath, Inc. v. Muhurkar*, 935 F.2d 1555, 1564-65, 19 USPQ2d 1111, 1117-18 (Fed. Cir. 1991).

With respect to claims reciting polynucleotides, "an adequate written description of a DNA ... 'requires precise definition, such as by structure, formula, chemical name, or physical properties, ...'" *Regents of the University of California v. Eli Lilly and Co.*, 119 F.3d at 1566, 43 USPQ2d at 1404 (quoting *Fiers v. Revel*, 984 F.2d at 1171, 25 USPQ2d at 1606). The written description requirement is met for a claimed polynucleotide if the polynucleotide is completely described in the specification by its chemical structure, *i.e.*, nucleotide sequence. *See Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1332, nt. 7, 65 USPQ2d 1385, 1398, nt. 7 (Fed. Cir. 2003) ("Indeed, Amgen's patents appear to satisfy the sequence requirement in *Eli Lilly* insofar as Figure 6 of the patents expressly discloses the complete (albeit slightly incorrect) sequence of human genomic EPO DNA and the encoded DNA.") and *Ex parte Reinherz*, 2002 WL 31003016, *2 (unpublished) (BPAI 2002) ("The best way of complying with the written description requirement, perhaps the only way, is to set forth the precise sequence of nucleotides that make up the claimed genetic material.").

In the absence of a proper *prima facie* case, an applicant who complies with the other statutory requirements is entitled to a patent. *See In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). On appeal to the Board, an appellant can overcome a rejection by showing insufficient evidence of a *prima facie* case by the Examiner. *See Id.*

B. 35 USC §112, First Paragraph - Enablement

The specification of a patent must describe the claimed invention in sufficient detail to enable any person skilled in the relevant art to make and use the full scope of the claimed invention without undue experimentation. *See* 35 USC §112, first paragraph ("The specification shall contain a written description of the invention, and of the manner and process of making and using it, ... to enable any person skilled in the art ... to make and use the same ....") and *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984). Whether a claim is sufficiently enabled by a disclosure in a specification is a question of law based on underlying factual inquiries, and is determined as of the date that the patent application was first filed, *i.e.*, its effective filing date. *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1371, 52 USPQ2d 1129, 1134, 1135 (Fed. Cir. 1999); *In re Hogan*, 559 F.2d 595, 604, 194 USPQ 527, 535 (CCPA 1977). Enablement may be satisfied by combining what is disclosed in a specification with what is known in the prior art. *In re Strahilevitz*, 668 F.2d 1229, 1232, 212 USPQ 561, 563-64 (CCPA 1982).

Although not explicitly set forth in the statute, enablement may be found where some experimentation (even a considerable amount) is required, so long as the

experimentation is not "undue." *Ex parte Forman*, 230 USPQ 546, 547 (BPAI 1986); *see also In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (J. Miller concurring) (CCPA 1977); and *In re Rainer*, 347 F.2d 574, 577, 146 USPQ 218, 220-221 (CCPA 1965). The Federal Circuit, adopting the analysis set forth in *Forman*, has enumerated several factors which may be considered in determining whether claims require that one skilled in the art perform undue experimentation in order to practice the claimed subject matter: breadth of the claims; predictability or unpredictability of the art; relative skill of those in the art; state of the prior art; nature of the invention; working examples; amount of guidance; and quantity of experimentation necessary. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). These factors are merely illustrative, not mandatory; they provide a general framework for analysis. *Enzo Biochem v. Calgene Inc.*, 188 F.3d at 1371, 52 USPQ2d at 1136; *Amgen, Inc. v. Chugai Pharm. Co.,* 927 F.2d 1200, 1213, 18 USPQ2d 1016, 1027 (Fed. Cir.), *cert. denied*, 502 U.S. 856 (1991).

In fact, enablement may still be present when an application contains no working examples or when prophetic examples are used. *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d at 1576-77, 224 USPQ at 414 ("Use of prophetic examples, however, does not automatically make a patent non-enabling.") and *In re Strahilevitz*, 668 F.2d at 1232, 212 USPQ at 563 ("Nevertheless, as acknowledged by the board, examples are not required to satisfy section 112, first paragraph.").

The Examiner has the burden to set forth a *prima facie* case by establishing a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). To comply with the how-to-use prong of the enablement requirement it is sufficient that

one method is disclosed in the specification that is commensurate in scope with the claimed invention. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Similarly, a single statement of utility within the specification that conveys how-to-use the claimed invention is sufficient to satisfy that prong of the enablement requirement. *In re Johnson*, 282 F.2d 370, 373, 127 USPQ 216, 219 (CCPA 1960).

On appeal to the Board, an appellant can overcome a rejection by showing insufficient evidence of *prima facie* lack of enablement. *See In re Oetiker*, 977 F.2d at 1445, 24 USPQ2d at 1444.

SUMMARY OF THE ARGUMENT

The Examiner improperly focused her written description analysis on unclaimed elements. In doing so, the Examiner (1) failed to consider the invention ***as claimed*** and completely ignored certain other claims; (2) failed to evaluate the ***relevant*** descriptions of the claimed invention that are in the specification; and (3) failed to apply the correct legal precedent. The Examiner also made factual errors on which the rejections are predicated. Consequently, the Examiner failed to make out a *prima facie* case of lack of written description.

The so-called "new ground of rejection" under the enablement provision of 35 USC §112, first paragraph is nothing more than the ***withdrawn*** utility rejections masquerading under a different section of the statute. Accordingly, the rejection should be reversed at least for the reasons already of record in Appeal Brief 1. The rejection is also untenable for the additional reasons that the Examiner (1) failed to construe the claims as written; (2) failed to consider the disclosure in view of the claims as written;

and (3) failed to apply the relevant law. Thus, the Examiner also failed to make out a *prima facie* case of lack of enablement.

ARGUMENT

POINT I

THE EXAMINER FOCUSED ON AN UNCLAIMED NAME AND AN UNCLAIMED FUNCTION AND REJECTED A PHANTOM SET OF CLAIMS

The Examiner is fixated upon Appellants' disclosure to the PTO that the polypeptide designated as SEQ ID NO:1 in the application was incorrectly ***named***. (*See* July 31, 2001 Response, p. 4, Ins. 11-19 and Paper No. 19, p. 6, Ins. 6-22). This myopic fixation on the name of SEQ ID NO:1 blinded the Examiner to the claims, *as written*, and the description in the specification supporting such claims and resulted in reversible error.

The first error is that the Examiner has not identified any authority that supports a conclusion that merely misnaming a compound is sufficient to establish a lack of written description. Moreover, even if there were such authority, ***a name is not claimed***, a polynucleotide corresponding to a described sequence identifier (*i.e.*, SEQ ID NO) is, and the Examiner has never addressed (except for entering the claim amendments set forth in the July 31, 2001 Response) the scope of the claims under appeal.

Thus, because the Examiner has not come to grips with the scope of the claims and the fact that a name is not claimed, the rejection must be reversed for this

reason alone. *See, e.g., Ex parte Böttcher*, 2002 WL 99677, *3 (unpublished) (BPAI 2002) ("To the extent that the rejection is predicated upon the written description requirement, ***we summarily reverse as we find the examiner has failed to specifically indicate what language in the claims is inadequately supported*** by the original specification.") (emphasis added) and *Ex parte Shi*, 2002 WL 230632, *2 (BPAI 2002) ("***The section 112, first paragraph, rejection plainly cannot be sustained*** to the extent that it is based upon a failure to comply with the written description requirement of this paragraph. This is because ***the examiner has not even identified the here claimed subject matter which is thought to be offensive to the written description requirement.***") (emphasis added).

The second error is that the Examiner, still focusing on the misnaming issue[4/], construed the claims to recite functional limitations that simply are not there:

> ***Even though the structure of the enzyme remains the same***, the name of the enzyme connotes a specific ***function*** to the enzyme and its use. Monooxygenases incorporate one hydroxyl group into their substrates whereas dioxygenases incorporate two atoms of dioxygen into their substrates, as discussed above. ***Even though both enzymes transform carotene to retinal, each reaction is different, leading to different intermediates and use of different co-factors***, as described.

(Paper No. 19, p. 6, lns. 13-18) (emphasis added).

Apparently, the Examiner construed one or more of the nucleic acid, primer, probe, kit or method claims to recite an intermediate enzymatic reaction in the

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4/ Applicants have explained the "misnaming issue" in full on page 7 of this Supplemental Appeal Brief and in Appeal Brief 1, which is hereby incorporated into the present Supplemental Appeal Brief in full. *See* Appeal Brief 1, pp. 13-14.

pathway leading from carotene to retinal. That, however, is ***not*** the claimed invention. ***Not*** one claim under appeal recites such a reaction pathway step. Indeed, ***not*** one claim under appeal recites a function. The claims are expressly limited to polynucleotide sequences and primers for such sequences[5/], whose chemical structures are fully disclosed in the Figures (*see e.g.*, Figures 3 and 4) and the Sequence Listing filed with the application.

Fundamentally, it is legal error to read a limitation into a claim that is ***not*** there. *In re Priest*, 582 F.2d 33, 37, 199 USPQ 11, 15 (CCPA 1978) *citing In re Prater*, 415 F.2d 1393, 1405, 162 USPQ 541, 551 (CCPA 1969) ("We have consistently held that no 'applicant should have limitations of the specifications read into a claim where no express statement of the limitation is included in the claim.'"); *Ex parte Bowles*, 23 USPQ2d 1015, 1017 (BPAI 1991) ("Therefore, in the instant case, while we have made every effort to liberally interpret the claims in light of the specification, ***it would be error on our part to infer or read into these claims any limitations from the specification.***") (emphasis added); and *Specialty Composites v. Cabot Corp.*, 845 F.2d 981, 988, 6 USPQ2d 1601, 1606 (Fed. Cir. 1988) ("In sum, the understanding of the art, the language of the other claims, the specification and the prosecution history, all show that the term 'plasticizer' in the claim language is not restricted to external plasticizers. ***The district court erred in reading this limitation into the claims before us.***") (emphasis added).

The Examiner did not – and could not - identify, which claim or claims

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5/ As set forth In Appendix I, the claims on appeal also include claims to probes, host cells, vectors and test kits incorporating a nucleic acid sequence encoding a polypeptide of SEQ ID NO:1, methods for introducing such a cDNA into a host cell and kits for amplifying polynucleotides using primers (SEQ ID NOs: 8, 9 and 10) defined in the specification.

under appeal contain(s) the functional or reaction pathway limitations, which form the basis of the rejection. In fact, the Examiner's only apparent consideration of the actual claim language was a single sentence that identified a structure, namely SEQ ID NO:1:

> The claims are drawn to DNA encoding a polypeptide of SEQ ID NO:1.

(Paper No. 19, p. 6, ln. 6).

Thus, the Examiner's own claim construction, which correctly identified one of the claimed *structures*, belied the erroneous written description analysis focusing on an ***unclaimed*** *function*. Where, as here, a rejection was based on a claim limitation that is not affirmatively recited nor inherently part of the claim, the rejection must be reversed.

In contrast to the phantom claims construed by the Examiner, the claims, ***as written***, all recite complete nucleotide structures described in Figures 3 and 4 (*e.g.*, SEQ ID NOs: 1, 2, 8, 9 and 10) and in the Sequence Listing filed concurrently with the application. The Table below identifies each sequence recited directly or through dependence for each appealed claim:

Claim No.	Sequence Identifier Recited
6, 10-15, 19-27	SEQ ID NO:1
28-32	SEQ ID NO:2
34, 36	SEQ ID NO:8
34, 36	SEQ ID NO:9
35, 36	SEQ ID NO:10

As noted above, the complete structures of SEQ ID NOs: 1, 2, 8, 9 and 10 are set forth in the application. *See, e.g.*, Figures 3 and 4 and the Sequence Listing filed with the application. The specification also describes SEQ ID NO:2 as a cDNA

sequence with a length of 3090 base pairs (excluding the polyA tail beginning at position 3073) having 132 base pairs of 5'-nontranslating sequence, a coding sequence of 1578 base pairs and a 3'-nontranslating sequence of 1380 base pairs. (Specification, p. 4, lns. 14-18). Furthermore, Example 4 describes how the full length cDNA was cloned. (*Id.*, p. 18, ln. 13 – p. 22, ln. 29). The specification also describes the chemical structure of the polypeptide sequence derived from the coding region of SEQ ID NO:2. This amino acid sequence is described as having 526 amino acid residues. (*Id.*, p. 4, lns. 20-22).

In view of the foregoing, under the *Lilly* case and its progeny, because each claim is limited to a complete structure, which structure is fully disclosed in the specification, each claim is in compliance with the written description requirement. *See also* the PTO's own SYNOPSIS OF APPLICATION OF WRITTEN DESCRIPTION GUIDELINES, in particular Example 8 concluding that a claim reciting: "An isolated and purified nucleic acid comprising SEQ ID NO:2." complies with the written description requirement.

In sum, because the Examiner (1) read into the claims functional and reaction pathway limitations not recited therein; (2) failed to consider the claims as written; and (3) failed to consider the properly construed claims in light of the specification, the Examiner failed to make out a *prima facie* case. For these reasons alone, the rejection should be reversed.

POINT II

THE EXAMINER ERRONEOUSLY APPLIED AN ENABLEMENT STANDARD TO REJECT THE CLAIMS FOR LACK OF WRITTEN DESCRIPTION AND FAILED TO CONSIDER DESCRIPTIONS IN THE SPECIFICATION THAT SHOW POSSESSION OF THE CLAIMS AS WRITTEN

As noted above, the Examiner focused on the name/function of the polypeptide sequence encoded by the claimed polynucleotides, notwithstanding what is actually claimed. In doing so, the Examiner again erred by using an ***enablement*** standard to reject the claims for lack of ***written description***.

In making the rejection, the Examiner observed that the specification does not disclose "how to use the claimed invention" and that the specification "only describes assaying a polypeptide" for β,β-carotene 15,15'-dioxygenase activity.

> The specification only describes ***how to use*** the claimed invention as a dioxygenase and not as a monooxygenase. Also, the specification only describes assaying a polypeptide for β,β-carotene 15, 15'-dioxygenase activity and not for β,β-carotene monooxygenase activity. ***Therefore***, at the time of filing, ***a skilled artisan would not recognize*** from the specification ***that applicants were in possession of a polypeptide with β,β-carotene 15, 15'-monooxygenase activity***.

(Paper No. 19, p. 6, ln. 19 – p. 7, ln. 2) (emphasis added).

As noted above, enablement and written description are separate and distinct statutory bases for rejection. *In re Barker*, 559 F.2d at 593, 194 USPQ at 474. Thus, the elements of a *prima facie* case for enablement and written description are different. By focusing exclusively on the elements of an enablement rejection – *e.g.*,

"how to use the claimed invention" (Paper No. 19, p. 6, lns. 19-20) – the Examiner ignored the elements of a *prima facie* written description rejection.

Here, the record is clear that the Examiner's rejection relied on a two-fold analysis that ignored the legal standards under the written description requirement for a *prima facie* case. The Examiner's entire rejection rested on the theory that because the specification did not describe "***how to use***" a monooxygenase and because the specification "only describes assaying" for β,β-carotene 15,15'-dioxygenase activity, the claimed invention was not described. Thus, what factual analysis was of record, was completely irrelevant to a written description analysis.

When an Examiner fails to set forth a *prima facie* case, an applicant who complies with the other statutory requirements is entitled to a patent. *See In re Oetiker*, 977 F.2d at 1445, 24 USPQ2d at 1444. For this reason also, the rejection should be withdrawn. In this regard, we also note that when an Examiner fails to set forth a *prima facie* case, it is ***not*** the Board's job to carry out the analysis that the Examiner did not. *Ex parte Matsumura*, 1996 WL 1771396, *9 (BPAI 1996) (unpublished) ("Again, the examiner merely concluded without making underlying factual findings and/or pertinent explanations. What effort did the examiner make to determine that there is no adequate disclosure for the feature referred to? What basis and which facts caused the examiner to come to his conclusions? It is also uncertain whether the examiner was referring to the written description requirement or the enablement requirement of 35 USC §112, first paragraph, which are distinctly different from each other. ***With respect to all of these uncertainties, we decline to speculate as to what the examiner had in mind and***

reiterate that it is not the rol of the Board to conduct patent xamination in the first instance.") (emphasis added).

What was relevant here, and what the Examiner should have, but did not consider, was whether the claimed polynucleotides were sufficiently described in the specification so that one skilled in the art could have distinguished them from others. *See e.g., Regents of the University of California v. Eli Lilly and Co.*, 119 F.3d at 1568, 43 USPQ2d at 1406 ("In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus.").

Notwithstanding that the Examiner's analysis focused on elements of the specification that were not claimed, which, as discussed above, alone is sufficient basis to reverse the rejection, whether or not the specification describes ***how to use*** the *claimed* invention as a dioxygenase or a monooxygenase is irrelevant to whether the *claimed* invention is adequately described. A written description analysis ***must*** focus on the ***structure*** of the ***claimed*** invention and whether the ***claimed*** structure is sufficiently described to show possession by the applicants at the time the application was filed. *Regents of the University of California v. Eli Lilly and Co.*, 119 F.3d at 1566, 43 USPQ2d at 1404.

Here, the Examiner's only apparent consideration of the description of the *claimed* invention was her admission that (1) the claims were drawn "to DNA encoding a

polypeptide of SEQ ID NO:1;" (2) the "structure of the enzyme remains the same;" and (3) the enzyme transforms carotene to retinal. (Paper No. 19, p. 6, lns. 6, 13, and 16-17).[6/] The Examiner appears to have ignored Figures 3 and 4 (as well as the Sequence Listing filed with the application), which show the complete polypeptide and polynucleotide structures for SEQ ID NOs: 2 and 1, respectively. The Examiner also appears to have ignored Example 4, which, *e.g.*, describes the use of recombinantly expressed SEQ ID NO:1 in a transactivation assay for detecting retinoic acid produced after β-carotene cleavage:

> 90 of the above pools were tested for activity in a transactivation assay based on the detection of retinoic acid which is produced in eukaryotic cells after β-carotene cleavage.

(Specification, p. 21, lns. 25-26).

For this additional reason, the rejection should be reversed.

6/ In view of the Examiner's withdrawal of the utility and enablement rejections set forth in Paper No. 14 and the Examiner's concession in Paper No. 19 that SEQ ID NO:1 "transforms carotene to retinal," there is no dispute that the application does describe at least one function that is correlated with the structure of SEQ ID NO:1.

POINT III

THE EXAMINER'S OMNIBUS REJECTION COMPLETELY IGNORES THE PRIMER/PROBE/TEST KIT CLAIMS (CLAIMS 12-15 AND 34-36) AND THE METHOD/HOST CELL CLAIMS (CLAIMS 19-27), WHICH ARE SEPARATELY PATENTABLE IN VIEW OF UNANALYZED DISCLOSURES IN THE SPECIFICATION

A. Claims 12-15 and 34-36

The evidence of record clearly shows that Examiner treated all the claims as standing or falling together even though the claims were of different scope including claims directed to isolated nucleotides or polynucleotides (claims 6, 10, 11 and 28-30), primers (claims 12 and 34-36), a probe (claim 13), test kits (claims 14 and 15), methods (claims 19-25), host cells (claims 26, 27 and 32) and vectors (claim 31). Such an omnibus rejection is clearly prohibited under the PTO's own internal rules and clearly falls short of the required *prima facie* case. *See* MPEP §707.07(d) at 700-113 ("A plurality of claims should ***never*** be grouped together in a common rejection, unless that rejection is equally applicable to all claims in the group.") (emphasis added). The Examiner ***never*** considered the primer, probe, test kit, method, host cell, and vector claims and ***never*** considered whether the so-called analysis actually set forth in the Office Action applied to these claims. For this additional reason, the rejection should be withdrawn.

An additional error in the rejection was the failure of the Examiner to consider the primer/probe/test kit claims, namely claims 12-15 and 34-36, separate from the rest of the claims on appeal. Claims 12-15 and 34-36 recite subject matter distinct from "DNA encoding a polypeptide of SEQ ID NO:1" – apparently the only claimed

subject matter considered by the Examiner. ("The claims are drawn to DNA encoding a polypeptide of SEQ ID NO:1.") (Paper No. 19, p. 6, ln. 6).

For example, claims 12 and 34-36 (the "primer claims") recite various "primers" (claims 12, 34 and 35) or kits incorporating primers (claim 36) used for amplifying a gene or a polynucleotide encoding the polypeptide of SEQ ID NO:1. There is no evidence of record that the Examiner considered a ***primer*** for SEQ ID NO:2 (the polynucleotide sequence encoding SEQ ID NO:1) separately from the DNA encoding SEQ ID NO:1. More particularly, there is no evidence that the Examiner considered SEQ ID NO:8, SEQ ID NO:9 or SEQ ID NO:10 – the polynucleotide primer sequences actually recited in claims 12 and 34-36 – separately from the claims "drawn to DNA encoding a polypeptide of SEQ ID NO:1." But that was the Examiner's burden. *See,* MPEP §2163 at 2100-163 ("Claim construction is an essential part of examination. Each claim ***must*** be ***separately analyzed*** and given its broadest reasonable interpretation in light of and consistent with the written description.").

Because there is a complete lack of evidence that the Examiner even considered, let alone "separately analyzed" claims 12-15 and 34-36 from the claims "drawn to DNA encoding a polypeptide of SEQ ID NO:1," the Examiner failed to meet her burden of setting forth a *prima facie* case of lack of written description.

B. Claims 19-27

Claims 19-25 are directed to a "method of introducing a cDNA coding for a polypeptide of SEQ ID NO:1 into a host cell" and claims 26 and 27 are directed to host cells made according to the method recited in, *e.g.*, claim 19. Again, there is ***no evidence*** that the Examiner construed claims 19-27 separately from the claims "drawn

to DNA encoding a polypeptide of SEQ ID NO:1." There is ***no evidence*** that the Examiner compared the scope of the construed claims to the scope of description in the application. And, there are simply no "express findings of fact," which support the rejection of claims 19-25. Accordingly, the Examiner did not meet her burden of setting forth a *prima facie* case of lack of written description for claims 19-27. *See, e.g., In re Oetiker*, 977 F.2d at 1445, 24 USPQ2d at 1444 and MPEP §2163.04 I at 2100-173 ("In rejecting a claim, the examiner must set forth express findings of fact which support the lack of written description conclusion .... These findings should: (A) Identify the claim limitation at issue; and (B) establish a *prima facie* case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed.").

For all of these reasons, the rejection of claims 12-15, 19-27 and 34-36 should be reversed.

POINT IV

THE EXAMINER'S "NEW" ENABLEMENT REJECTION IS NOTHING MORE THAN THE WITHDRAWN REJECTIONS MASQUERADING UNDER A DIFFERENT STATUTORY PROVISION

Stripped of all pretense, the rejection of claims 6, 10-15, 19-32 and 34-36 for lack of enablement rests solely on the Examiner's continued fixation on the name assigned to SEQ ID NO:1. In view of Appeal Brief 1, the Examiner conceded that her utility and enablement rejections were insufficient, declined to go forward with the appeal, and withdrew both rejections. The so-called "new" enablement rejection is

nothing more than an attempt to revisit the old rejections that have been considered and withdrawn. Applicants should not have to relitigate the same issue multiple times until the Examiner comes to grip with the admitted fact that misnaming a compound alone is insufficient to support a rejection under §101 or §112, first paragraph. It is time to move on.

In the present rejection (Paper No. 19), the Examiner characterized the alleged deficiency in the description as not teaching "***[h]ow to use*** SEQ ID NO:1 as a monooxygenase" and that "[w]ithout such guidance, the ***experimentation*** left to those skilled in the art is ***undue***." (Paper No. 19, p. 4, lns. 16-17 and p. 5, lns. 20-21) (emphasis added). In Paper Nos. 11 and 14, however, the alleged deficiency was a lack of utility under §101 and a lack of enablement under §112, first paragraph because "one skilled in the art clearly would not know ***how to use*** the claimed invention so that it would operate as intended without ***undue experimentation***." (Paper No. 11, p. 4, lns. 2-4 and Paper No. 14, p. 3, lns. 19-21) (emphasis added).

The current rejection and the rejection set forth in Paper Nos. 11 and 14 are the same. The arguments set forth in Appeal Brief No. 1 are equally applicable here and are incorporated by reference as if recited in full herein. These arguments were sufficient to require withdrawal of the rejections in Paper No. 14 and nothing has changed since then – neither the claims nor the rejection has been altered. Accordingly, for all of the reasons set forth in Appeal Brief 1, the so-called "new" enablement rejection should be reversed.

POINT V

THE ENABLEMENT REJECTION FAILS TO FOLLOW THE REQUIRED *WANDS* ANALYSIS DEFINED BY THE LEGAL PRECEDENT IDENTIFIED BY THE EXAMINER

The Examiner admitted that any enablement rejection must begin with a consideration of the *Wands* factors. *See* Paper No. 19, p. 3, lns. 13-15. As noted above, the *Wands* factors provide a framework for the enablement analysis. Depending on the facts, not all of the *Wands* factors need be considered to sustain an enablement rejection. Where, as here, the rejection fails to consider ***any*** of the Wands factors with respect to the *claimed* invention, the rejection cannot be sustained.

A rejection based on enablement requires a claim-by-claim analysis. *See, e.g., Ex parte Jochim*, 11 USPQ2d 1561, 1562 (BPAI 1989) (Board separately construing the scope of claims 29 and 30 directed to a hybridoma and an antibody, respectively.). As noted above, the Examiner's ***only*** attempt at construing the breadth of the claims was her all-encompassing statement that the claims are "drawn to DNA encoding a polypeptide of SEQ ID NO:1." This statement is, at best, incomplete, and is completely irrelevant to claims 12-15, 19-27, 31, 32 and 34-36, which are separately patentable for this reason as well.

Moreover, the Examiner completely ignored the *Wands* factors of (1) the amount of guidance provided by the specification with respect to the claimed invention, (2) working examples, (3) nature of the invention, and (4) predictability/unpredictability of the art.

With respect to the remaining three *Wands* factors, namely an analysis of the relative skill of the art, the state of the prior art, and the quantity of experimentation

required, the Examiner's focus was on what was known with respect to mono- and dioxygenases. *See* Paper No. 19, p. 3, ln. 20 – p. 5, ln. 21 ("Even though monooxygenases are well known in the art, ***it would not have been obvious to one skilled in the art to practice the instant invention*** as a monooxygenase. At the time of filing, one skilled in the art would not have know to use SEQ ID NO:1 as a monooxygenase because the specification very clearly states that SEQ ID NO:1 is a β,β-carotene 15,15'-dioxygenase.") (emphasis added).[7/]

Thus, the Examiner, at best, paid lip service to the *Wands* factors and failed to apply any of them appropriately to the invention as claimed. For this reason also, the rejection should be reversed.

7/ We also note that whether or not it "would have been obvious" to practice the claimed invention as a monooxygenase is irrelevant to whether the claims are enabled by the specification. Reliance on a §103 standard obscures the Examiner's burden to consider the evidence as whole – not what would have been obvious. *Ex parte Cox*, 2002 WL 1801332, *3, nt. 2 (unpublished) (BPAI 2002) (Board criticizes a deficient rejection for only paying lip service to the *Wands* factors, without analyzing the claims and written description). Simply put, "obviousness" is not the standard under §112, first paragraph. Accordingly, because the Examiner used the wrong standard, the rejection should be reversed for this reason as well. *See, e.g., Ex parte Levengood*, 28 USPQ2d 1300, 1301 (BPAI 1993) (reversing a §103 rejection because Examiner applied wrong standard); *Ex parte Lee*, 1996 WL 1805906, *2 (unpublished) (BPAI 1996) (reversing a §112, first paragraph rejection because Examiner applied wrong standard); and *Gambro Lundia AB v. Baxter Healthcare Corp.*, 110 F.3d 1573, 1577-1578, 42 USPQ2d 1378, (Fed. Cir. 1997) (reversing lower court's decision applying an improper obviousness standard in a §102(f) derivation analysis).

POINT VI

THE EXAMINER FIXATED UPON THE NAME OF SEQ ID NO:1 AND FAILED TO CONSIDER THE CLAIMED INVENTION AND SUPPORTING DISCLOSURE IN THE SPECIFICATION

The Examiner's enablement rejection rested exclusively on her apparent belief that misnaming a claimed compound in the specification was sufficient to support a *prima facie* case of non-enablement. The Examiner, however, offered no case to support the proposition that misnaming a molecule is fatal to enablement. The single dimension of the rejection suggests a *per se* rule was applied by the Examiner. It is well settled, however, that there are no *per se* rules of patentability. *See Ex parte Gates*, 1997 WL 33121875, *3 (unpublished) (BPAI 1997) ("Applicants and the examiner each apparently argue for the application of a *per se* rule which results in either a reversal or affirmance of the rejection depending on whose *per se* rule we apply. However, ***no such per se rules exist***. Whether the description requirement has been satisfied depends on the particular facts of each case. The specification as a whole must be analyzed to determine whether the written description allows a person of ordinary skill in the art to recognize that applicants invented the claimed subject matter.") (emphasis added) and *Ex parte Yang*, 2002 WL 32102463, *3 (unpublished) (BPAI 2002) (Board chastises examiner for applying a *per se* rule that eliminates the need for fact specific analysis of the claims and the prior art in an obviousness rejection) and *In re Ochiai*, 71 F.3d 1565, 1570-1572, 37 USPQ2d 1127, 1132-33 (Fed. Cir. 1995). Because the Examiner applied a *per se* rule, the rejection should be reversed for this reason alone.

Far from the *per se* rule adopted by the rejection, the Examiner was ***required*** to construe the claims ***as written*** and then determine whether each rejected claim was supported by disclosure in the application. *See e.g.*, MPEP §2164.01 at 2100-178 ("Any analysis of whether a particular claim is supported by the disclosure in an application ***requires*** a determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention."). The Examiner's sole claim construction is set forth in a single sentence:

> The claims are drawn to DNA encoding a polypeptide of SEQ ID NO:1.

(Paper No. 19, p. 3, ln. 20).

Instead of construing each of the claims as written, such as for example, claim 6, which recites:

> 6. An isolated nucleic acid sequence encoding a polypeptide of SEQ ID NO:1.

the Examiner analyzed alleged admissions by the Applicants regarding the function of the polypeptide of SEQ ID NO:1:

> Applicants have admitted to the misidentification of the function of the enzyme represented by SEQ ID NO:1. Applicants have admitted that post filing studies revealed SEQ ID NO:1 to be a β,β-carotene

> 15,15'-monooxygenase rather than a β,β-carotene 15,15'-dioxygenase. (internal citations omitted).[8/]

(Paper No. 19, p. 4, lns. 1-4).

The Examiner then summarily concluded that the specification does not "teach" a monooxygenase or how to use a monooxygenase:

> ***The specification does not teach*** that SEQ ID NO:1 is ***a β,β-carotene 15,15'-monooxygenase nor how to use*** SEQ ID NO:1 as ***a β,β-carotene 15,15'-monooxygenase***. Therefore, the breadth of the claims is much larger than the scope enable[d] by the specification.

(*Id.*, lns. 4-7) (emphasis added).

The Examiner further observed that monooxygenases and dioxygenases both transform carotene to retinal, but through different intermediate reactions:

> ***Even though both enzymes transform carotene to retinal, each reaction is different***, leading to ***different intermediates*** and ***use of different cofactors, transition metals, flavin and/or pteridine***. For example, monooxygenases utilize FAD and the second atom of oxygen is reduced to water either by the substrate themselves or by a co-substrate reductant. (internal citations omitted).

(*Id.*, lns. 18-22) (emphasis added).

8/ As already set forth in Appeal Brief 1 (pp. 14-15), the ***only*** error in the specification identified by the Appellants is the ***name*** associated with the polypeptide designated as SEQ ID NO:1. All other properties disclosed in the specification, including the polypeptide and polynucleotide sequences, the identified substrate for SEQ ID NO:1 (β-carotene) and the reaction product (retinal) are correct.

The Examiner's analysis misses the point. The question is not whether the specification describes how to make or use a monooxygenase. Rather, the question is whether ***the claims as written*** are enabled by a disclosure in the specification. ***None*** of the claims recite a function for the *polypeptide* sequence set forth in SEQ ID NO:1. ***None*** of the claims even claim the *polypeptide* sequence set forth in SEQ ID NO:1. Rather, ***all*** of the claims recite *polynucleotide* sequences or *primer* sequences and methods, vectors, host cells, and test kits incorporating such sequences. Furthermore, the Examiner admitted in Paper No. 19 that the specification discloses a use for SEQ ID NO:1, namely transformation of carotene to retinal:

> Even though both enzymes [mono- and di-oxygenases] transform carotene to retinal ....

(Paper No. 19, p. 4, ln. 18).

The specification describes at least three uses for the polypeptide encoded by SEQ ID NO:2, namely (1) a participant in the pathway leading to production of vitamin A; (2) cleavage of carotene molecules; and (3) the production of transgenic plants.[9/] Moreover, in view of the withdrawal of the utility rejection, the Examiner admitted that the specification described a use for the claims under appeal. And, as developed in Appeal Brief 1, these asserted uses stand unrebutted. (*See* pp. 20-21).

It cannot be disputed that the structure of the polypeptide sequence (SEQ ID NO:1) is fully disclosed. *See e.g.*, Figure 3 and SEQ ID NO:1. ***It cannot be disputed*** that the structure of the claimed polynucleotide sequences are fully disclosed.

9/ These uses were previously brought to the Examiner's attention (see July 31, 2001 Response, p. 5, lns. 1-16) and Appeal Brief 1, pp. 20-21.

See, e.g., Figure 4 and SEQ ID NOs: 2, 8, 9 and 10. ***It cannot be disputed*** that the application discloses how to make the full length cDNA (SEQ ID NO:2) using specific primers (SEQ ID NOs: 8, 9 and 10) and how to express it. *See, e.g.*, Example 4. ***It also cannot be disputed*** that the specification discloses that the polypeptide whose sequence is set forth in SEQ ID NO:1 is a participant in the pathway leading to the production of vitamin A and is able to cleave carotene molecules; and that the polynucleotide whose sequence is set forth in SEQ ID NO:2 may be used to produce transgenic plants. And, the Examiner has not contended – and cannot contend – that one skilled in the art could not make either the polynucleotide sequence (SEQ ID NO:2) or the encoded polypeptide (SEQ ID NO:1) given the description of the sequences in the application. Nothing more is required. Indeed, Example N of the PTO's own training materials confirms that nothing more is required to comply with the enablement provision:

> Claim 1[10/] is limited to a single DNA sequence and any 15 mer thereof. ***Since the state of the art is such that it would have been routine to make the DNA given the sequence, it certainly would not require undue experimentation to make the DNAs claimed in claim 1. Furthermore, the specification clearly shows how to use the full length DNA to produce algernin and the 15 mers to obtain the full length DNA.*** Therefore, it would not require undue experimentation to make or use the DNAs of claim 1 and no enablement rejection should be made.
>
> ***For claim 2[11/], the analysis is similar even though the genus is very large.*** There are at least 1.26

10/ Claim 1 of the hypothetical claim recites:

1. An isolated cDNA that comprises the following DNA sequence and encodes algernin:
   ATG ...
   or fragments thereof that are at least 15 nucleotides in length.

11/ Claim 2 of the hypothetical claim recites:

> $X10^{21}$ embodiments of the claim, but each embodiment can be readily identified using the genetic code, synthesized using conventional methods, and used in the manner taught in the specification without undue experimentation.

(*See*, Example N: DNA, TRAINING MATERIALS FOR EXAMINING PATENT APPLICATIONS WITH RESPECT TO 35 USC §112, FIRST PARAGRAPH ENABLEMENT CHEMICAL/BIOTECHNICAL APPLICATIONS ("Enablement Training Materials"), pp. 57-59) (emphasis added).

Thus, the PTO's own analysis for a claim limited to a specific DNA sequence (hypothetical claim 1), which is analogous to present claim 28, which recites an isolated polynucleotide sequence (SEQ ID NO:2), focused on two issues:

(1) The state of the art is such that if a DNA sequence is provided, it is a routine matter to make it; and

(2) The specification in Example N described how to use the cDNA to make algernin.

Here, just as in Example N, the complete structure of SEQ ID NO:2 was disclosed in Figure 3 and in the Sequence Listing. And, the PTO admitted that the state of the art was such that it was a routine matter to make a polynucleotide sequence if its structure is described. Here also, just as in Example N, the specification described how to make the polypeptide (SEQ ID NO:1) from the cDNA (SEQ ID NO:2). *See* Example 4. Nothing further is required to enable a claim to a specific polynucleotide sequence (*i.e.*, claim 28). And, the analysis for a claim to a set of degenerate polynucleotide

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2. An isolated DNA that encodes the following amino acid sequence for algernin:
Met ...
or fragments thereof that are at least 15 nucleotides in length.

sequences that encode a specifically described polypeptide sequence is similar to the above analysis. Thus, present claim 6, which is analogous to hypothetical claim 2 of Example N is likewise enabled.[12/]

Had the Examiner conducted the proper analysis – the analysis set forth above – the inescapable conclusion is that the present claims are enabled. Because, however, the Examiner failed to carry out the proper analysis, the rejection should be reversed for this reason as well.

POINT VII

THE EXAMINER'S REJECTION AMOUNTS TO A NOVEL REQUIREMENT TO ENABLE WHAT IS NOT CLAIMED AND IMPERMISSIBLY SHIFTS THE BURDEN TO THE APPELLANT TO DEMONSTRATE ENABLEMENT IN THE ABSENCE OF A *PRIMA FACIE* CASE

As set forth above, it was the Examiner's burden to set forth a *prima facie* case of lack of enablement. *In re Oetiker*, 977 F.2d at 1445, 24 USPQ2d at 1444. A *prima facie* case of enablement must include a construction of the claims and a factual analysis of why the specification does not disclose how to make and/or use the claimed invention without undue experimentation. When a rejection does not meet these basic requirements, it cannot be sustained. *See Ex parte Shi*, 2002 WL at 23063, *2 (BPAI 2002) (unpublished) ("To the extent that the rejection is based upon a failure to comply with the enablement requirement, the rejection still cannot be sustained. In this regard,

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12/ Likewise, enablement for the rest of the primer, probe, test kit, method, vector, and host cell claims flows from the disclosure of the cDNA sequence (SEQ ID NO:2) and the polypeptide sequence encoded by it (SEQ ID NO:1), recognition of several disclosed uses for such sequences, and the admitted knowledge in the art for making polypeptide and polynucleotide sequences from their disclosed structures.

we emphasize that the examiner has failed to carry his initial burden of advancing acceptable reasoning inconsistent with enablement.").

The Examiner did not construe the claims as written, which alone was sufficient to reverse the rejection. And, the Examiner's analysis of the scope of disclosure of the specification was limited to whether or not the specification described how to make or use a β,β-15,15'-monooxygenase. But, as noted above, ***none*** of the claims are so limited.

It is well settled that when a claim does not recite a specific function or use, enablement is met when an application describes any function or use that is commensurate in scope with the claim. *See* MPEP §2164.01(c) at 2100-180 ("...when a compound or composition is not limited by a recited use, ***any*** enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use.") (emphasis added).

By rewriting Appellants' claims to include a monooxygenase function and then analyzing the specification in view of these phantom claims, the Examiner impermissibly shifted the burden to Appellants to prove that an unclaimed element is enabled. Such a burden shifting flies in the face of the clear requirement that the Examiner has the initial burden to set forth a *prima facie* case in view of the claims *as written*.

Although it is not Appellants' burden, we note that there are at least three unrebutted uses for the claimed invention described in the specification, namely use in

the Vitamin A pathway (Specification, p. 2, lns. 21-22), cleavage of carotene (Specification, p. 11, lns. 15-20, p. 21, lns. 23-27, and p. 25, lns. 9-12), and construction of transgenic plants (Specification, p. 12, lns. 6-12). Yet another use of the polynucleotide sequence, SEQ ID NO:2, is the making of the polypeptide sequence, SEQ ID NO:1. (*See, e.g.*, Example 4). In view of these clear descriptions of how to use both SEQ ID NOs:1 and 2, the how-to-use prong of the claimed invention is met.

The PTO acknowledged – and the Examiner did not contend otherwise - that at least as of November 5, 1996 (roughly four years *before* the U.S. filing date of the present application), when the Enablement Training Materials were published, it was a routine matter to synthesize polynucleotides and polypeptides from an identified sequence:

> The state of the prior art is also such that given a specific sequence it is routine to synthesize DNA and proteins.

(Enablement Training Materials, p. 59).

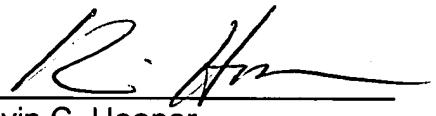
In view of the PTO's own admission that it was within the skill of the art to make DNA and protein if the polynucleotide and polypeptide sequences were described, it must be admitted that given the express description of the polynucleotide sequence of SEQ ID NO:2 and the polypeptide sequence of SEQ ID NO:1 that these sequences could have been made without undue experimentation by one skilled in the art. Thus, when the specification is analyzed with a view toward what is actually claimed, the how-to-make prong of the claimed invention is also met.

Accordingly, notwithstanding the Examiner's impermissible burden shifting, a proper enablement analysis unquestionably demonstrates that the claims, ***as written***, are in compliance with the enablement provision of §112, first paragraph. For this reason as well, the rejection should be reversed.

CONCLUSION

For all of the foregoing reasons, it respectfully is submitted that the Examiner has failed to make out a *prima facie* case of lack of written description and non-enablement and hence the rejection of claims 6, 10-15, 19-32, and 34-36 should be reversed.

Respectfully submitted,

By: 

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APPENDIX I

6. An isolated nucleic acid sequence encoding a polypeptide of SEQ ID NO:1.

10. An isolated nucleic acid sequence according to claim 6 wherein the nucleic acid is a deoxyribonucleic acid.

11. An isolated nucleic acid sequence comprising an antisense ribonucleic acid, which binds to the nucleic acid sequence according to claim 6.

12. A primer for amplifying a gene coding for the polypeptide of SEQ ID NO:1 which primer comprises a fragment of the nucleic acid sequence according to claim 6.

13. A probe for detecting a gene coding for the polypeptide of SEQ ID NO:1 which probe comprises a fragment of the nucleic acid sequence according to claim 6.

14. A test kit for amplifying and/or detecting a gene or a fragment thereof coding for the polypeptide of SEQ ID NO:1 wherein the test kit comprises at least one primer according to claim 12.

15. A test kit for amplifying and/or detecting a gene or a fragment thereof coding for the polypeptide of SEQ ID NO:1 wherein the test kit comprises at least one probe according to claim 13.

19. A method for introducing a cDNA coding for the polypeptide of SEQ ID NO:1 into a host cell comprising introducing a cDNA coding for the polypeptide of SEQ ID NO:1 into a vector suitable for the host cell and introducing the vector into the host cell.

20. A method according to claim 19 wherein the host cell is a plant cell.

21. A method according to claim 19 wherein the host cell is a prokaryotic cell.

22. A method according to claim 19 wherein the host cell is a yeast cell or a fungal cell.

23. A method according to claim 19 wherein the host cell is an alga cell.

24. A method according to claim 19 wherein the host cell is a mammalian cell.

25. A method according to claim 24 wherein the mammalian cell is a human cell.

26. A host cell obtained by the method of claim 19.

27. A host cell according to claim 26 which comprises a cDNA coding for the polypeptide of SEQ ID NO:1 obtained from another species.

28. An isolated polynucleotide which encodes the polypeptide of SEQ ID NO: 1 comprising SEQ ID NO:2.

29. An isolated polynucleotide according to claim 28 which consists essentially of SEQ ID NO: 2.

30. An isolated polynucleotide according to claim 28 which consists of SEQ ID NO: 2.

31. A vector comprising the polynucleotide of SEQ ID NO: 2.

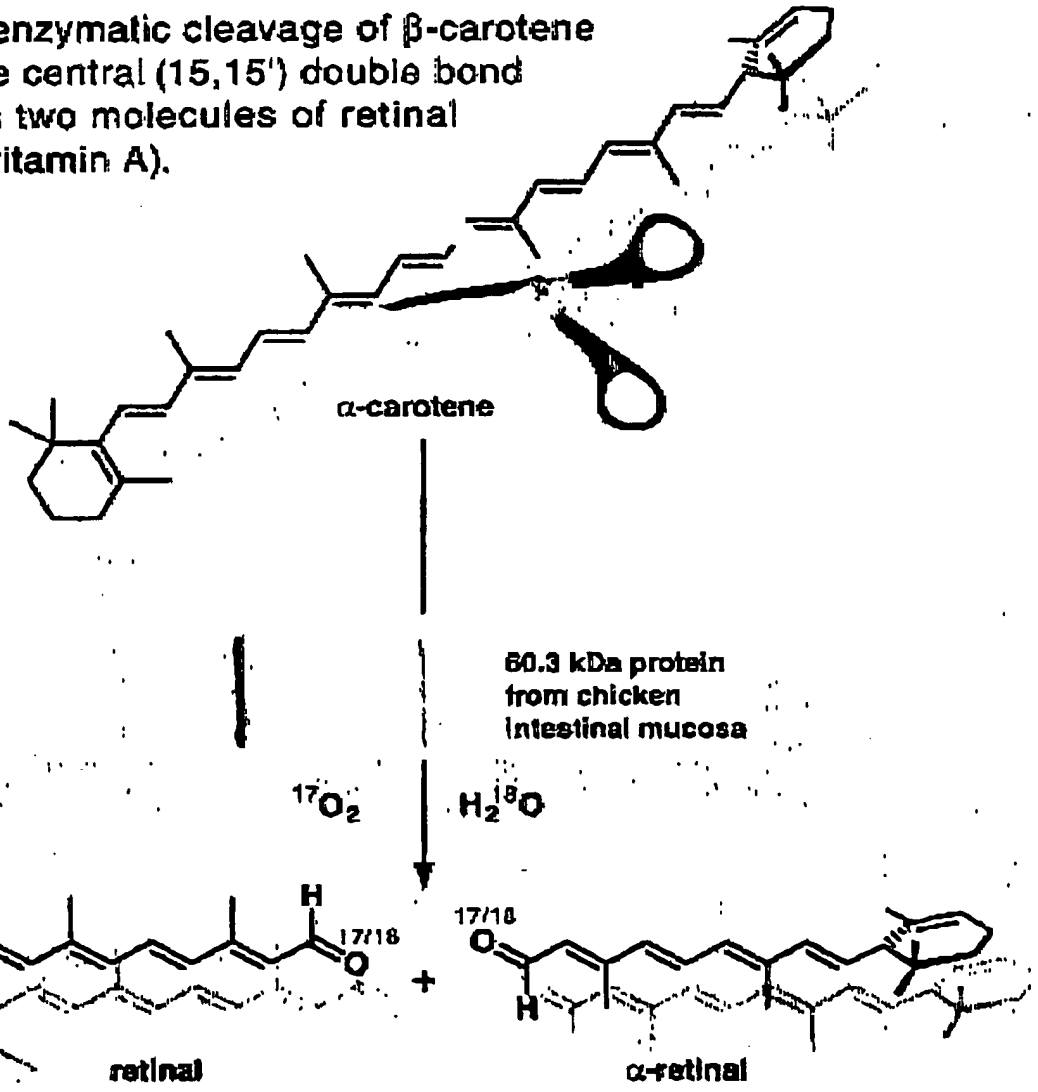
32. A host cell transformed with the vector of claim 31.

34. A primer set for amplifying a polynucleotide encoding the polypeptide of SEQ ID NO:1 comprising SEQ ID NO:8 as a 5' primer and SEQ ID NO: 9 as a 3' primer.

35. A primer set for amplifying a polynucleotide encoding the polypeptide of SEQ ID NO:1 comprising a polyT/Not reverse primer and SEQ ID NO:10 as a forward primer.

36. A kit for amplifying and/or detecting a polypeptide or fragment thereof encoding the polypeptide of SEQ ID NO:1 comprising at least one primer selected from the group consisting of SEQ ID NOs:8, 9, and 10.

The enzymatic cleavage of β-carotene at the central (15,15') double bond gives two molecules of retinal (provitamin A).



Investigation of the reaction mechanism with α-carotene as a substrate revealed a monooxygenase pathway since both $^{17}O_2$ and $H_2{}^{18}O$ are incorporated into the metabolites.

Find out more on the following pages.

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The Reacti n Mechanism of the Enzyme-Catalyzed Central Cleavage of β-Car tene to Retinal**

Michele G. Leuenberger, Caroline Engeloch-Jarret, and Wolf-D. Woggon*

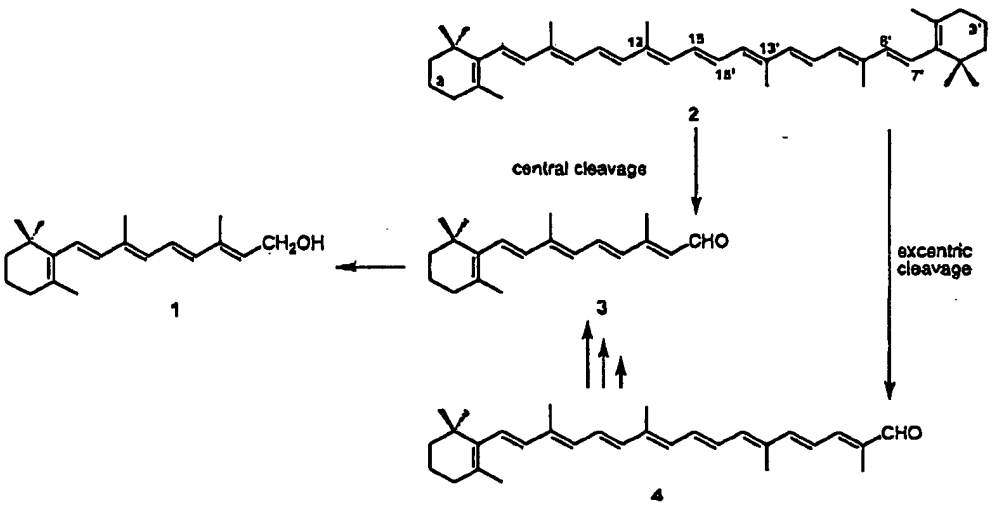
Dedicated to Professor Synnøve Liaaen-Jensen

Although it has been known since 1930 that vitamin A or retinol (1) derives in vivo from β-carotene (2),[1] the enzymatic origin of β-carotene cleavage was only shown in 1965 when Olson and Hayaishi reported the identification of in vitro activity of an enzyme from rat liver and rat intestine. This enzyme catalyzes the central cleavage of 2 to retinal (β-retinal, 3; Scheme 1).[2] Later an alternative, probably less significant, pathway was discovered involving excentric cleavage of 2 to yield apo-carotenals such as 4, which are subsequently degraded to 3.[3]

During the last 35 years many groups have tried unsuccessfully to purify the enzyme catalyzing the central cleavage of β-carotene 2,[4] and quite a number of mechanistic investigations have been published which involved crude enzyme preparations or in vivo experiments.[2, 5, 6] Solid information, however, could only be obtained for two aspects of the problem: The enzyme requires molecular oxygen, and the central cleavage proceeds stoichiometrically to yield approximately two moles of retinal from one mole of β-carotene.[7] Most of the other experiments regarding the incorporation of oxygen from water and concerning the metal involved in catalysis can be valued as inadequate.[8] Nevertheless the enzyme that catalyzes the central cleavage of 2 was termed β-carotene 15,15'-dioxygenase (EC1.13.11.21) and often the enzyme was believed to be an iron dioxygenase.[2, 9]

Recently we[10a] and others[10b] have been able for the first time to identify the protein which catalyzes the central cleavage. We have developed a purification protocol for the enzyme from chicken intestinal mucosa and it has become possible to overexpress the functional 60.3 kDa protein in BHK (baby hamster kidney) cells.[10a,c] We have also investigated the substrate specificity of the enzyme with the aim of identifying a nonsymmetrical carotenoid that could be utilized for investigation of the mechanism.[11] This aspect was mainly overlooked in earlier work, we believe. However, only the use of a nonsymmetrical carotenoid as a substrate to yield different aldehydes can provide exact information on the incorporation of oxygen from water and/or air into cleavage products and, hence, distinguish a monooxygenase from a dioxygenase mechanism.

Substrate specificity studies revealed three nonsymmetrical carotenoids, 5–7, that are readily cleaved by the enzyme (40–50 % of the yield obtained for 2 under standard conditions) to furnish the corresponding aldehydes, for example, 5 → 3 and 8 (Scheme 2). α-Carotene 5 was chosen as the best candidate because it was available in isomerically pure form, and it was expected that aldehydes 3 and 8 would behave similarly in the subsequent reactions that would be required for mass spectrometry (MS) analysis of the distribution of the labeled oxygen in both cleavage products. In this context it is important to note that aldehydes such as 3 and 8 are not directly suitable for isotopic analysis of an oxygen label in the carbonyl group[12] because this label easily exchanges with the medium at the pH value of incubation (pH 7.8).[13] Thus, we decided for a combined enzyme assay with addition of horse liver alcohol dehydrogenase (HLADH) to reduce 3 and 8 in situ to the corresponding alcohols retinol (1) and α-retinol (9). Alcohols 1 and 9 are also unsuitable for tandem gas chromatography/mass spectrometry (GC-MS) analysis because both eliminate water. Thus, after quenching of the incubation and high-pressure liquid chromatography (HPLC) purification of alcohols 1 and 9 (Figure 1a), derivatization to the silyl ethers 10 and 11 was required (Figure 1b).



Scheme 1. Enzymatic cleavage of β-carotene 2. Routes to the formation of retinol 1.

Control experiments revealed that the rates of reduction of 3 and 8 are the same. Exchange of the carbonyl oxygen of 3 with the buffer medium was investigated under conditions similar to the incubation conditions, that is, 3 was added to a

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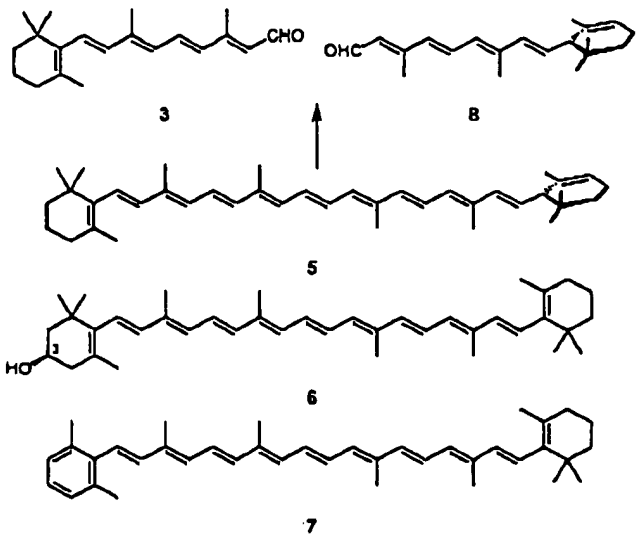


Scheme 2. Nonsymmetrical substrate analogues of the enzyme that catalyzes the central cleavage of 2. Cleavage of α-carotene 5 to form the aldehydes 3 and 8.

solution of HLADH in $H_2{}^{18}O$ as slowly as it would be produced by enzymatic cleavage of 5 (3.5 nmol h^{-1}). According to MS analysis of the retinyl silylether 10, exchange of the ^{18}O label between 3 and $H_2{}^{18}O$ is <5%.

For the decisive incubation experiment with 5 the native enzyme was employed due to its favorable turnover, which is ≈2.5 times higher than the hexahistidine-tailed protein overexpressed in BHK cells. Highly enriched oxygen sources, such as 85% $^{17}O_2$ and 95% $H_2{}^{18}O$, were used. GC-MS analysis of the silylethers 10 and 11 with the focus on the molecular ion

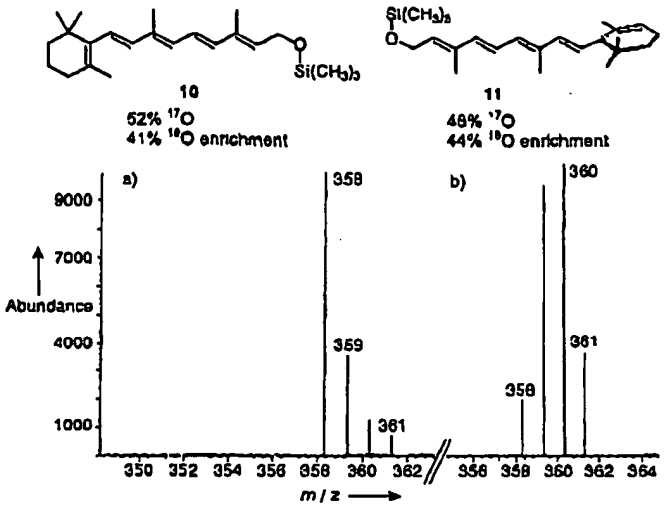


Figure 2. Mass spectra: a) silylether 10 with natural abundance of oxygen isotopes; b) $^{17}O/^{18}O$-enriched 10 from incubation of 5 in the presence of $^{17}O_2$ and $H_2{}^{18}O$.

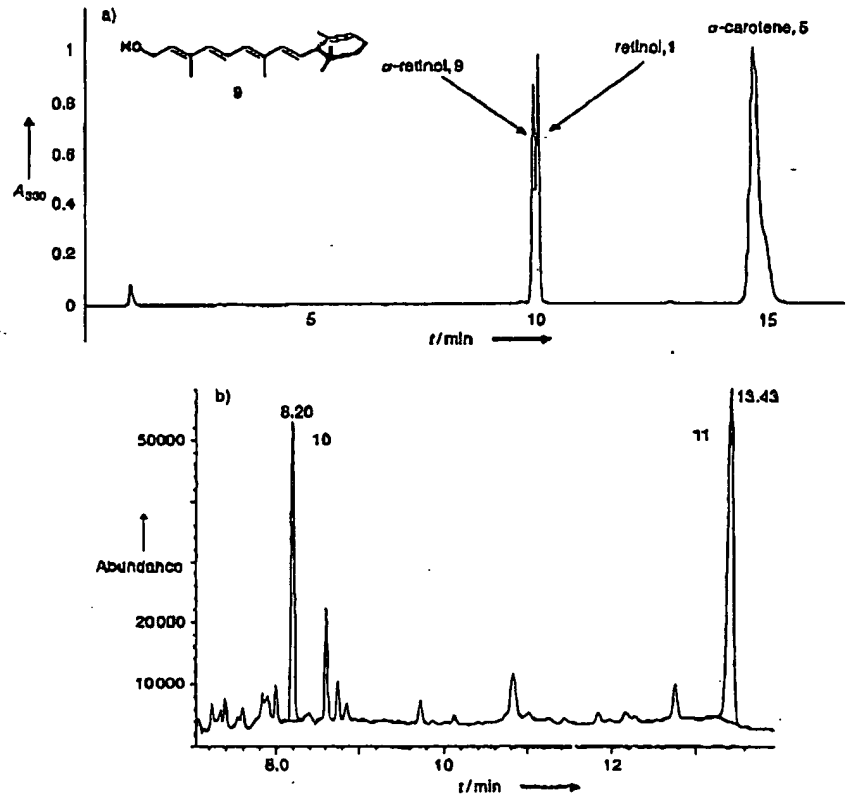


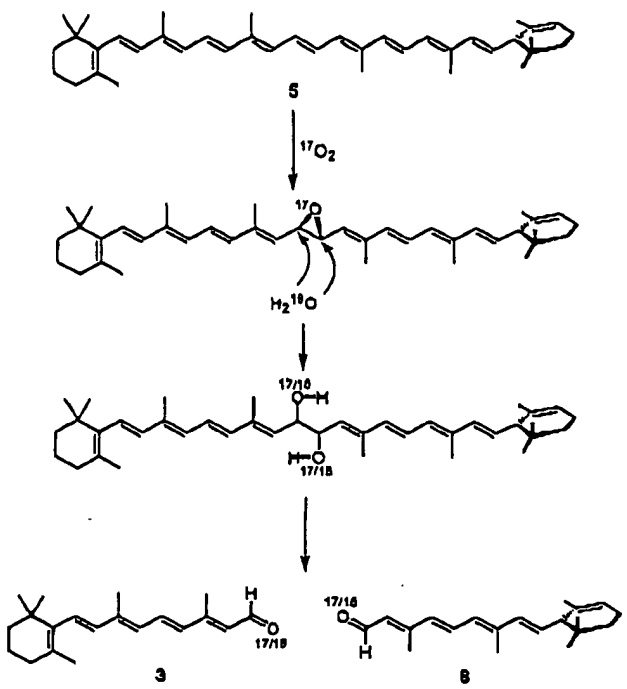
Figure 1. a) HPLC trace recorded after incubation of α-carotene 5; b) GC trace for 10 and 11, the silylethers of the metabolites of 5.

area revealed, within experimental error, equal enrichment of the ^{17}O and ^{18}O label in both derivatives of metabolites 3 and 8 (Figure 2). This result proves for the first time the incorporation of one ^{17}O atom of molecular oxygen and the concomitant incorporation of one ^{18}O atom from labeled water.

Accordingly, and in contrast to earlier beliefs, the reaction mechanism of enzymatic central β-carotene cleavage is not in agreement with a dioxygenase-catalyzed procedure. A dioxygenase mechanism ([2+2] cycloaddition to the central C15–C15′ double bond, followed by fragmentation of the intermediate dioxetane) would require the incorporation of one complete oxygen molecule into the product aldehydes and the absence of any ^{18}O label originating from the labeled water.

Experimental evidence provided here accounts for a monooxygenase-type mechanism, as shown in Scheme 3, in which the first step is an epoxidation of the central double bond of 5. This is followed by unselective ring opening with water and final diol cleavage to yield the aldehydes 3 and 8.

Another small experimental detail agrees with the monooxygenase mechanism. Given the enrichment of the labels in both oxygen



Scheme 3. The reaction mechanism of the central cleavage of α-carotene 5 catalyzed by the 60.3 kDa cytosolic monooxygenase purified from chicken's intestinal mucosa. The mechanism for β-carotene 2 is thought to be analogous.

sources, one would expect, in case of quantitative O-incorporation, the following isotopic enrichments for **10** and **11**: 10% ^{16}O, 42.5% ^{17}O, and 47.5% ^{18}O. Experimentally, however, one finds 5–8% higher ^{17}O enrichment than calculated along with the correspondingly lower ^{18}O enrichment (systematic deviation $\leq \pm 2\%$). This difference can be explained by assuming that ^{17}O-labeled water originating from $^{17}O_2$ cleavage in the active site "dilutes" the $H_2{}^{18}O$ oxygen source in situ.

The nature of the metal complex involved in O_2 cleavage and epoxidation still has to be elucidated. At present it is only certain that this first step of carotene metabolism is not a P450-catalyzed reaction because the heme-thiolate chromophore ($\lambda_{max} \sim 415$ nm) is absent in the purified protein (broad absorption without fine structure between 200–280 nm) as well as in the overexpressed enzyme. Interestingly the monooxygenase mechanism resembles, at least in part (the epoxidation), the mechanism we previously proposed for a supramolecular enzyme model catalyzing the regioselective cleavage of **2** and **7**.[14]

Experimental Section

Enzymatic reaction conditions: α-carotene **5** ((6′*R*)-β,ε-carotene) was obtained from F. Hoffmann-La Roche (Basel) and stored at −18 °C. A stock solution of **5** in benzene (10 mM) was freshly prepared. In a glass vial the stock solution of **5** (40 μL), α-tocopherol solution (50 μL, 43 mg mL^{-1} in hexane), and tween 40 solution (200 μL, 400 μL in 10 mL acetone) were evaporated with a gentle stream of N_2 in a heated block (45 °C). Tricine buffer ($H_2{}^{18}O > 95\%$; 1 mL, 150 mM; pH 7.8, 45 °C) was added and the solution was gently mixed until almost complete solubilization. The substrate solution was added to a 25-mL flask containing tricine buffer ($H_2{}^{18}O > 95\%$; 3.5 mL, 150 mM; pH 7.8), glutathione (12 mg), sodium cholate (1 mg), and nicotinamide adenine dinucleotide, reduced form (NADH; 50 mg). The mixture was cooled to −180 °C while connected to a high-vacuum line (3×10^{-2} mbar) and then degassed three times. Finally labeled molecular oxygen (> 85% $^{17}O_2$; 20 mL; 2.15 bar) was condensed on the surface of the frozen solution (−180 °C). The mixture was allowed to warm up, and after reaching 25 °C the system was allowed to equilibrate over 30 min. In a separate flask the enzyme purified by hydrophobic interaction chromatography (HIC)[15] (from ≈ 10 g mucosa of one chicken's duodenum) was dissolved in tricine buffer ($H_2{}^{18}O > 95\%$; 500 μL, 150 mM; pH 7.8). HLADH (80 μL, 11.1 mg mL^{-1}, 2.9 U mg^{-1} protein; Fluka AG, Buchs) was added. Argon was passed through the solution for 30 min/25 °C to remove $^{16}O_2$. The enzymatic reaction was started by adding this solution to the substrate solution (final concentration of **5**: 80 μM). After incubation for 7.5 h at 37 °C in the dark, the reaction was quenched by addition of acetonitrile (4 mL). The mixture was extracted three times with chloroform (4 mL) and the collected organic phases were evaporated to dryness.

Purification by HPLC: The residue obtained as described above was separated by analytical HPLC (LiChrospher 100 RP-18 5 μm, dimensions: 125 × 4.6 mm, 25 °C, flow rate: 1 mL min^{-1}, eluents and gradients: 100% solution of acetonitrile/1% NH_4OAc_{aq} (1:1) → 100% solution of acetonitrile/*i*PrOH (1:1) over 10 min, the eluent remained the same for 5 min, then → 100% solution of acetonitrile/1% NH_4OAc_{aq} (1:1) over 2 min; diode array detector at 330 nm for **1** (R_t = 10.0 min) and **9** (R_t = 9.9 min), and 455 nm for **5** (R_t = 14.9 min). The mixture of retinoids was collected, evaporated, and concentrated in a 100-μL vial.

Silylation and GC-MS analysis: The mixture of the two products **1** and **9** was dissolved in hexane (5 μL). A syringe was purged several times with *N*,*O*-bis(trimethylsilyl)acetamide and then directly used to take an aliquot (1 μL) of the solution of **1** and **9**. Thus, silylation occurred in the syringe followed by immediate splitless injection (285 °C) into the GC column (cross-linked 5% phenylmethyl silicone, dimensions: 25 m × 0.2 mm, film thickness: 0.33 μm, 30 s purge delay; temperature program: 150 °C → 250 °C at 25 °C min^{-1} and then constant at 250 °C).[16] Selected ion monitoring analysis (electron ionization, 70 eV) was pursued for the [*M*$^+$] regions of the spectra for **10** (R_t = 8.2 min) and **11** (R_t = 13.4 min). The retention times of the retinoids **1** and **9** on HPLC and of the silyl derivatives **10** and **11** on GC were confirmed by injection of authentic material.

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Saddle-Shaped Six-Coordinate Iron(III) Porphyrin Complexes Showing a Novel Spin Crossover between $S = 1/2$ and $S = 3/2$ Spin States**

Takahisa Ikeue, Yoshiki Ohgo, Tatsuya Yamaguchi, Masashi Takahashi,* Masuo Takeda, and Mikio Nakamura*

Spin states of iron(III) porphyrins are controlled by the number and nature of axial ligands.[1] The coordination of nitrogen bases such as imidazole (HIm) and pyridine results in the formation of low-spin ($S = 1/2$) six-coordinate complexes. In contrast, anionic ligands such as Cl^- and F^- lead to the formation of five-coordinate high-spin ($S = 5/2$) complexes. Maltempo discussed a spin-admixed $S = 3/2$, 5/2 state on the basis of quantum mechanical calculations, and suggested that the $S = 3/2$ state is an important contributor to the spin state of certain bacterial heme proteins known as cytochromes c'.[2]

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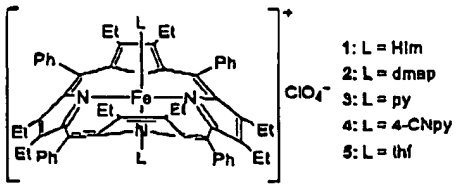
We and others recently reported that highly nonplanar (porphyrinato)iron(III) complexes with weak axial ligands show a quite pure intermediate spin state.[3, 4] The results were ascribed to the short $Fe-N_{por}$ bonds of the nonplanar porphyrin rings and the weak coordination ability of the axial ligands.[5] We therefore expected that the spin state of nonplanar $[Fe^{III}(oetpp)L_2]ClO_4$ (**1**–**5**) could change from



$[Fe(Oetpp)L_2]ClO_4$

the pure $S = 1/2$ to the pure $S = 3/2$ state as the axial ligand changes from strong HIm to weak THF; the order of the coordination ability is HIm > dmap > py > 4-CNpy > thf.[6] Of particular interest are the spin states of **2**–**4** because the axial ligands of these complexes are ranked between HIm and THF.

Table 1 lists the Mössbauer parameters, isomer shift (IS; relative to α-iron foil at 290 K), and quadrupole splitting (QS) measured at ambient and liquid nitrogen temperatures. The QS values for **1** and **2** at ambient temperature were within the range of low-spin complexes.[7] The IS and QS values for **4**

Table 1. Mössbauer parameters and spin state (S) of **1**–**5**.

		T [K]	IS [$mm\,s^{-1}$]	QS [$mm\,s^{-1}$]	Γ_1 [$mm\,s^{-1}$]	Γ_2 [$mm\,s^{-1}$]	S
1		297	0.18	1.82	0.24	0.25	1/2
		78	0.26	1.86	0.40	0.62	1/2
2		290	0.19	2.21	0.27	0.32	1/2
		80	0.26	2.31	0.55	0.89	1/2
3		290	0.32	2.76	0.27	0.29	3/2–1/2
		80	0.25	2.29	0.47	0.64	1/2
4	site A	295	0.37	3.26	0.32	0.33	3/2
	site A	80	0.57	3.03	0.47	0.47	3/2
	site B	80	0.20	2.70	0.64	0.64	1/2
5		290	0.41	3.65	0.32	0.26	3/2
		80	0.50	3.50	0.77	0.49	3/2

(0.37 and 3.26 $mm\,s^{-1}$, respectively) were close to those for **5** (0.41 and 3.65 $mm\,s^{-1}$); **5** has been fully characterized as the quite pure intermediate-spin complex.[4] Thus, from the viewpoint of Mössbauer spectroscopy, **1** and **2** are the low-spin complexes, while **4** is the intermediate-spin complex at ambient temperature. Figure 1 shows the Mössbauer spectra of **3** and **4** taken at ambient temperature and 80 K. The features change as the temperature is lowered. Complex **4** exhibited a new doublet (site B) below 230 K, and the relative intensities for this site increased on decreasing the temperature. The values for sites A and B are in the range of intermediate-spin and low-spin complexes, respectively, and both spin states co-exist at low temperature. This observation implies the occurrence of a novel spin-crossover process [Eq. (1)].[8, 9]

$$\text{low spin } (S = 1/2) \rightleftharpoons \text{intermediate spin } (S = 3/2) \qquad (1)$$

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